

STUDIES IN THE PIGMENTARY SYSTEM OF CRUSTACEA

III. LIGHT-INTENSITY AND THE POSITION OF THE DISTAL RETINAL PIGMENT IN *LEANDER ADSPERSUS*

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INTRODUCTION

While studying retinal pigment migration in a Mediterranean decapod, *Leander adspersus*, we noticed that the position of the distal pigment cells in the eyes of an animal kept on an illuminated black background was different from that in a shrimp maintained on a similarly-illuminated white background. Although it has been previously shown that the light-adapted condition of the distal retinal pigment in crustaceans is effected by a hormone having its origin in the eye-stalks (Kleinholz, 1936), it has been assumed that the position of the distal retinal pigment at any one time was to be found in either the light-adapted or the dark-adapted condition.

The preliminary observation mentioned above, if verified by further study, would indicate that the position of the retinal pigment could be graded between the limits of dark- and of light-adaptation, and that the gradation could be achieved by varying not only the intensity of light but also the color of the background. This suggests, furthermore, that the pigmentary reactions of the crustacean retina are not entirely determined by the intensity of incident light, but are possibly regulated to some extent by the intensity of reflected light, or by some relation between these two factors. Such a possibility is supported by the fact that Keeble and Gamble (1904), Brown (1936), and Sumner and Doudoroff (1937) have found the chromatic reactions of crustaceans and fishes to be determined by a similar relationship (the intensity of incident light/the intensity of reflected light).

We therefore decided to investigate the relation between light intensity and the position of the distal retinal pigment in the shrimp, *Leander adspersus*.

We are indebted to Professor R. Dohrn for the facilities granted us at the Stazione Zoologica in Naples. One of us (L.H.K.) takes this

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opportunity to thank the Committee for permission to occupy the Jacques Loeb Memorial Table.

MATERIALS AND METHODS

To reduce the error introduced by variations in the size of the eye among different individuals, only those shrimps measuring exactly 1.8 cm. from the tip of the rostrum to the posterior margin of the cephalothorax were used in these experiments. Two glass vessels, each about 10 inches in diameter and 5 inches deep, were prepared for the black and the gray backgrounds. The inside of one was covered with a melted mixture of paraffin-wax and lampblack; when this cooled it gave a uniform dull-black background. The gray background was prepared by first applying the black paraffin and then lining the inside of the dish with white-waxed tissue paper.

Ordinary electric-light bulbs of 25, 40, 50 and 100 watts were used as sources of illumination. In most of the series the bulbs were kept at a distance of 22 inches from the bottoms of the containers, but in two cases (series *B-3* and *A-4*) the position of the electric lamp was adjusted to give ratios which were desired as points on the scale. Exact quantitative measurements of light-intensity were not possible because of the lack of an appropriate photometer. Since only the ratios of incident to reflected light were sought, these were obtained quantitatively in a manner adequate for the purpose of these experiments by using a "Sixtus" photometer (Leica-model). The scale of this meter which expresses light-intensity in terms of seconds necessary for photographic exposure (the lower the numerical value of the reading the higher the intensity of light) gave relative values for the various intensities and backgrounds. The intensity of incident light was measured at the level of the bottom of the vessel. Each value for the intensity of reflected light was the average of 10 readings taken against the bottom of the empty container.

At the start of an experiment, sea water was poured into the dish to a level of 2.5 cm., 5 measured shrimps were introduced, and the temperature of the water taken. Twenty-four hours were allowed for retinal adaptation. At the end of 12 hours the temperature of the water was again taken and the water changed. In no case had the temperature risen more than 1.6°C. at the end of any twelve-hour interval. After 24 hours the eyes were fixed.

The animals were immersed in hot water (80°C.) for 10 seconds to fix the position of the retinal pigments. The eyes were then excised and transferred to a modified Bouin's solution (containing 7 parts of glacial acetic acid) for 24 hours. They were next placed successively in 70 per cent alcohol for 3 hours, in dioxane for 12 hours and in paraffin-wax

for 3-5 hours. Before the final embedding the eyes were oriented in the melted paraffin with the dorsal side uppermost (readily recognized by the black pigment-spot located at the junction of the retinal and non-retinal portions of the eye-stalk). Serial sections were cut at 20μ and were mounted unstained. Measurements of the position of the distal retinal pigment were made with an ocular micrometer through the middle of the eye, from the basement membrane to the proximal end of the distal pigment cells in 10 serial sections which included the dorsal pigment spot (Fig. 3). The structure of the eye of *Leander* is similar to that of *Palaemonetes* (Kleinholz, 1936).

In one experiment (Series I) the animals were illuminated from below by a 40-watt lamp, at a distance of 22 inches from the bottom of the clear glass container. The dish used for gray background was inverted over the container.

RELATION OF LIGHT-INTENSITY TO PIGMENT MIGRATION

The data from these experiments are arranged in Table I. Figure 1

TABLE I

Light-intensity and the position of the distal retinal pigment. Intensity of light was measured in relative rather than in absolute units (see under "Methods"). Series *A* were on the gray background, Series *B* on the black background, Series *D* in total darkness. *I*, intensity of incident light; *R*, intensity of reflected light; the ratio, I/R , was multiplied to give whole numbers; *P.P.*, distance of the proximal end of the distal pigment cells from the basement membrane in μ , and the average deviation within a series.

Bulb	Series	<i>I</i>	<i>R</i>	$\frac{I}{R} \times 1000$	<i>P.P.</i>
25	<i>A</i> -1	0.40	15.0	26	$295\mu \pm 17\mu$
40	<i>A</i> -2	0.25	6.3	39	$241\mu \pm 9\mu$
50	<i>A</i> -4	0.18	3.5	51	$190\mu \pm 18\mu$
100	<i>A</i> -3	0.125	2.0	62	$167\mu \pm 15\mu$
25	<i>B</i> -1	0.40	45.0	9	$372\mu \pm 18\mu$
40	<i>B</i> -2	0.22	13.0	17	$319\mu \pm 18\mu$
100	<i>B</i> -3	0.18	8.1	22	$311\mu \pm 24\mu$
—	<i>D</i>	—	—	—	$526\mu \pm 22\mu$

shows that, provided the background is kept constant, the amount of migration of the retinal pigment is directly proportional to the intensity of incident light (limited, however, by the total migration possible in passing from complete light- to complete dark-adaptation: about 360μ in the standard-size animals used here). The effect of backgrounds is shown by the different extents to which the distal pigment migrated in animals kept in the black and in the gray containers, but at the same intensities of incident light. The prolongations of the two curves meet

at a point — 515μ — where the positions of the distal pigment should be identical, regardless of the color of the background. Obviously, this theoretical position of the pigment should be found in the retinas of animals adapted to darkness. Measurement of the position of the retinal pigment in 7 dark-adapted eyes gave an average value of $526\mu \pm 22\mu$, in good agreement with the theoretical value.

When the data in the last two columns of Table I are plotted (Fig. 2), it becomes evident that, under the conditions of illumination used, the position of the distal pigment is in direct proportion to the ratio, $\frac{I}{R}$.

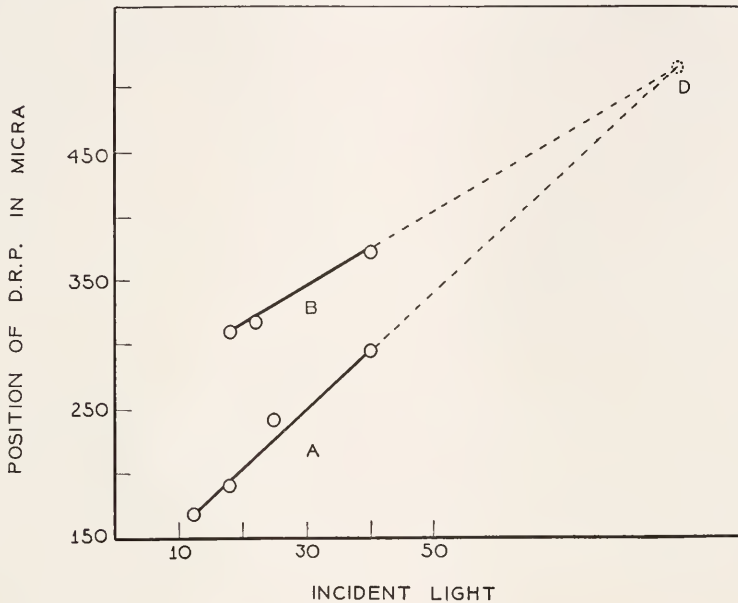


FIG. 1. The relation between intensity of incident light and position of the distal retinal pigment as measured from the basement membrane to the proximal end of the pigment cells. *A*, series on the gray background; *B*, series on the black background; *D*, theoretical position of the pigment in darkness (see text).

Whether the same direct relation would hold for the lower intensities of light, such as used by Brown (1936) in studying the chromatophoral behavior of a teleost, is not known.

Keeble and Gamble (1904, p. 354) found that color reactions were normal in crustaceans which were illuminated from below against white or black backgrounds, and that an implied dorsi-ventrality was therefore "not due to a permanently fixed structural difference in the two sides of the eye" but was produced "by some mobile structure which

takes up its position in response to the light conditions.”³ On examining sections of retinas in any particular stage of light-adaptation, it is seen (Figs. 4-7) that the pigment in the dorsal portion of the eye has always undergone a greater amount of migration (toward the basement membrane) than that in the ventral portion. But since migration of the distal pigment is under hormonal control (Kleinholz, 1936), the reason for the unequal position of this pigment is not clear. If the variation in position of the pigment of the dorsal and ventral regions of the retina is constant under all conditions of illumination, the inequality could be attributed to greater sensitivity of the pigment cells in the dorsal portion of the retina to the eye-stalk hormone. If, on

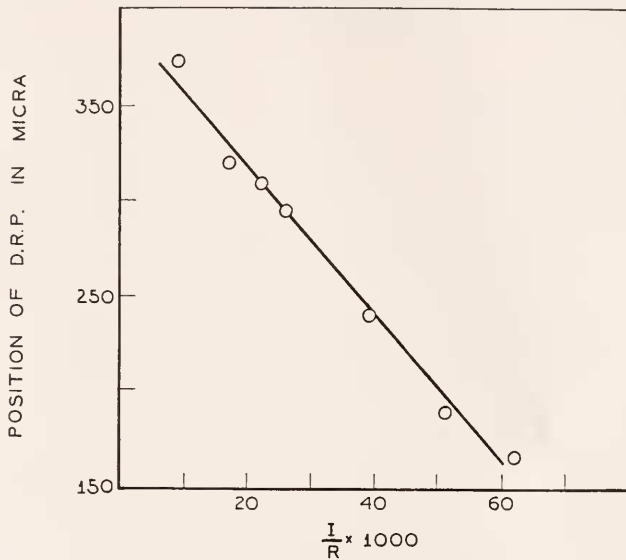


FIG. 2. The relation between position of the distal retinal pigment and the ratio, $\frac{\text{incident light}}{\text{reflected light}}$.

the other hand, this difference could be reversed by changing the method of illumination, the above explanation would not be valid; in such an event the greater amount of migration in the portion of the eye most strongly illuminated (toward the source of light) might be due to an additional direct effect of light on those cells. This point was

³ Butcher and Adelmann (1937) reported, however, that illumination of *Fundulus heteroclitus* from below against white and against black backgrounds did not result in the same color changes evoked in illumination from above, therefore implying a fixed structural difference in the retina. This difference apparently lies in the structure and distribution of the rods and cones in the dorsal and ventral portions of the retina (Butcher, 1937).

tested experimentally by subjecting animals to illumination from below against the gray background (see "Methods").

The results from this series are not entirely conclusive. Of 7 eyes studied 3 showed uniform migration of the pigment, the position of the

EXPLANATION OF PLATE I

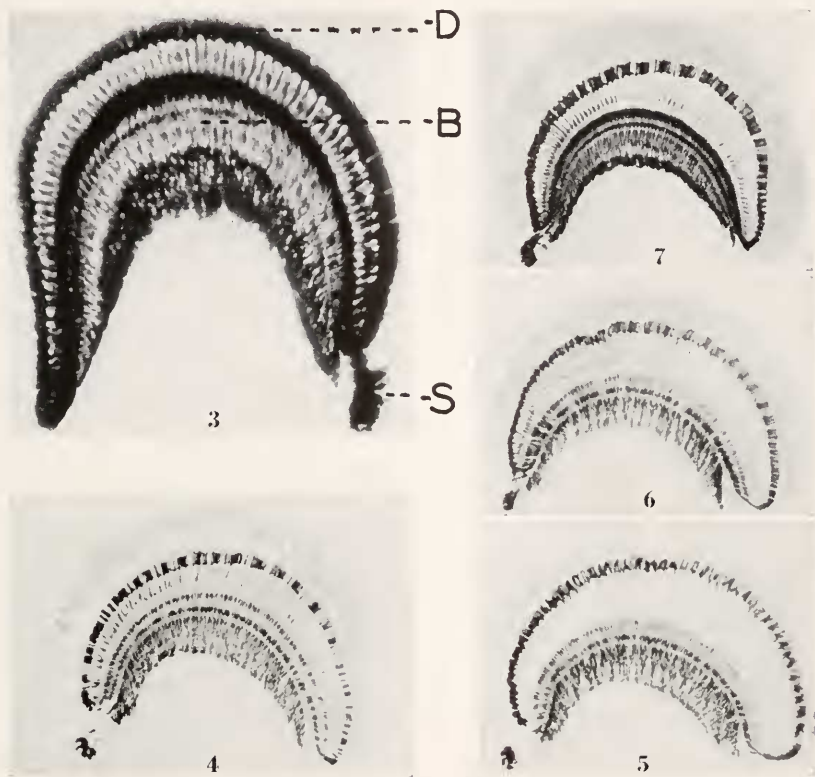


FIG. 3. An enlarged photograph of a completely light-adapted retina, showing the landmarks used in measuring the position of the distal pigment. *D*, distal pigment cells; *B*, basement membrane; *S*, dorsal pigment spot.

FIGS. 4 AND 5. Retinas from Series *A-1* and *B-1* respectively. The intensity of incident light was the same in both cases, but the backgrounds were different. Not only is the position of the distal pigment unequal in the two eyes, but that in the dorsal region of the retina has migrated to a greater extent toward the basement membrane.

FIGS. 6 AND 7. Retinas from Series *B-2* and *A-2* respectively.

distal cells being the same in the dorsal and ventral halves of the retina; in three others the distal retinal pigment had migrated to a greater extent in the dorsal portion of the eye; and one eye was irregular. Inasmuch as the pigment had migrated to a greater extent in the dorsal

portion of the retina in the eyes of all animals illuminated from above (except in series A-3 where the eyes were completely light-adapted) the fact that in some of the animals illuminated from below migration of the distal retinal pigment was uniform may indicate a slight direct response of those cells to light. This cannot be stated with any certainty, however, and requires further study.

DISCUSSION

It is generally stated that, within certain limits, chromatophoral activity in animals on particular backgrounds is largely independent of the intensity of incident light. Recent studies have been indicating a certain relation between the visual field and the chromatophore response. Thus, Sumner (1933) found that covering the lower halves of the eyes in *Fundulus parvipinnis* resulted in darkening of the animals even on white backgrounds, while darkening the upper half of the field of vision gave only a slight dispersion of melanophore pigment in fishes on white backgrounds. Hanström (1937) obtained similar results in opaquing the dorsal and ventral portions of the retinas of *Palaemonetes vulgaris*. Brown (1936) found that in the minnow, *Ericymba buccata*, "the average diameter of the melanophore masses (therefore the degree of darkening) appears to vary in a directly proportional fashion with the ratio, $\frac{\text{incident light}}{\text{reflected light}}$, which reaches the eyes of the fish."

All these results resolve themselves into a differential stimulation of dorsal and ventral retinal receptors. In *Leander* a similar condition must hold for the migration of the retinal pigment. The migration of the retinal pigment, however, is assumed to regulate the amount of light which arrives at any particular rhabdome. (In dim light the distal position of the pigment allows oblique rays to pass; in bright light oblique rays are excluded, only those rays parallel to the long axis of the ommatidium reaching the rhabdome.) The above experiments, therefore, do not determine whether the different extents to which the retinal pigment migrates in the dorsal and ventral halves of the eye contribute to the differential stimulation of dorsal and ventral photoreceptors or whether they are the result of such a differential stimulation.

SUMMARY

1. On a constant background the amount of migration of the distal retinal pigment in *Leander adspersus* is directly proportional to the intensity of incident light, within the limits of light- and of dark-adaptation.

2. Under the conditions of illumination used in these experiments the position of the distal retinal pigment is in direct proportion to the ratio, $\frac{\text{intensity of incident light}}{\text{intensity of reflected light}}$.

3. In all eyes, except those completely light- and totally dark-adapted, the pigment is found to migrate to a greater extent in the dorsal half of the retina. The reason for this variation in position of the pigment of the dorsal and ventral portions of the retina is not clear.

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